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Report on Doctoral Thesis submitted by **Mgr. Barbora SMIDOVA**

Thesis: The role of myeloid cells in mice infected with the neuropathogenic schistosome *Trichobilharzia regenti* (Study Programme: Parasitology; Research Supervisor: RNDr. Tomáš Macháček)

Conflict of interest statement

Firstly, as thesis reviewer, I confirm that I have NO bias or conflict of interest in relation to the work or the author of the thesis.

Overall assessment

After reviewing the thesis of Mgr. Barbora Smidova I conclude that the work contained therein is suitable for a Doctoral award and I recommend that the defence of the thesis takes place.

Brief summary and commentary on the thesis

The submitted doctoral thesis focusing on *Trichobilharzia regenti* and its interaction with the host by Mgr. Barbora Smidova investigates two specific areas of research: (i) the role and mechanisms of selected myeloid cells in the killing of *T. regenti* schistosomules and possible post-infection tissue repair; and (ii) evaluation of *T. regenti* as a possible candidate for alleviating the symptoms of experimental autoimmune encephalomyelitis (EAE), the mouse model of multiple sclerosis. The thesis is presented as three research papers, two published and one manuscript (presumably to be submitted), sandwiched between an Introduction (including Aims) and Discussion/Summary. Overall, the work done is novel and advances our understanding of the mechanisms by which the mouse clears the parasite in experimental infection, which replicates accidental infection.

The Introduction to the thesis is generally well written and interesting and successfully sets the scene for the rest of the thesis. This section demonstrates a broad understanding of the research area and is properly supported by relevant and up-to-date references. The Discussion/Summary, while perhaps a little short (see below), also brings the separate elements of the thesis together appropriately and discusses their importance to the field.

Importantly, the research student has generated a range of meaningful data, evidenced by their contribution to the research papers presented. The student has become proficient at a range of experimental techniques including, cell culture, flow cytometry, animal dissection, physiological scoring, immunohistochemistry, ELISA, DNA quantitation, gene expression analysis, and data analysis. They have also played a major part in the writing of the manuscript and published papers, appearing as third and first author on the latter, and first author on the additional manuscript. Overall, this contribution is commensurate with the requirements of a PhD.

I would like to take the opportunity to highlight the significance of the work in the field, particularly that conducted by the student published in the paper entitled “Mechanisms of the host immune response and helminth-induced pathology during *Trichobilharzia regenti* neuroinvasion in mice” published in PLoS Pathogens in 2022. The depth and breadth of this work and its overall excellence (evidenced by acceptance in this high-ranking journal) is such that it could only be achieved through significant collaboration amongst a team; the work of the candidate, highlighted in the author contributions, has clearly been instrumental to the success of the study and is commendable. The two additional first author contributions, while shorter in length and depth, add to the body of work overall and provide further insight into the mechanisms of parasite killing by the host and opportunities for immunomodulation as a therapeutic tool.

Specific comments on professional, linguistic and formal aspects of the thesis

Overall, the thesis is very well written and is structured in a coherent and logical fashion. Although some sentences could be better written, and there are several minor typographical errors throughout the thesis, this does not detract significantly from its overall quality. It would have been useful to have included a diagram or two in the introduction, including perhaps a life cycle diagram of the parasite which could also include migration routes in the definitive/accidental hosts. A diagram that also summarises the main findings of the thesis could also have been incorporated into the Discussion to support the text. Furthermore, some expansion of the Discussion to cover future avenues for research would also have been good to include.

Questions

Given that two of the three articles presented have been published in respected peer-reviewed journals (PLoS Pathogens, Parasitology), I do not propose to ask specific questions relating to them. However, the following general questions are provided to help stimulate discussion during the defence, along with a couple of specific questions relating to the yet to be published manuscript.

- 1) What do we know about the specific migration route/pattern of *T. regenti* in the mouse and its CNS and what (if any) host molecules exist to attract the parasite to migrate in this way?
- 2) Could you discuss/hypothesise on the possible role of excretory-secretory products (ESPs) produced by the *T. regenti* schistosomules during migration and during its colonisation of the CNS, on stimulation of myeloid cells including eosinophils and macrophages. How might such interactions be studied in the laboratory?
- 3) What other avenues of research did you attempt in your project and did they yield any preliminary data that is interesting but not included in the thesis?
- 4) Why did you not attempt to use schistosomules and/or their ESPs in the ETosis experiments? Is there a benefit to using homogenates? If so, and notwithstanding your comments about further validation being needed, how representative of the *in vivo* situation might this be?
- 5) In the Etosis experiments how did you control for the possibility that parasite DNA may influence the DNA quantitation?
- 6) What do you think are the most pressing outstanding questions relating to *T. regenti*-host immune interactions/tissue repair and how could they be investigated?

Finally, congratulations on the production of a very nice thesis.

Yours sincerely



Anthony John Walker
Professor of Cell Biology